



J1050 U.S. PTO

04-09-01

09807137-120902

JC04 Rec'd PCT/PTO 06 APR 2001

PATENTS

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09/807137

EXPRESS MAIL CERTIFICATION

Att'y's Docket No. 33148.004

"Express" Mail label number: EK594319063US

(A) Date of Deposit: April 6, 2001

I hereby certify that this transmittal letter and the papers and fees identified in this transmittal letter as being transmitted herewith are being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 C.F.R. 1.10 on the date indicated at (A) above and are addressed to the Assistant Commissioner of Patents, Washington, D.C. 20231

Name of Person mailing the above: Sonya Longo

Signature of Person mailing the above item

TRANSMITTAL LETTER TO THE UNITED STATES
DESIGNATED/ELECTED OFFICE (DO/EO/US)

International Application No.: PCT/EP99/07542
International Filing Date: 8 October 1999 (08.10.99)
Priority Date Claimed: 8 October 1998 (08.10.98)
Title of Invention: Method and Device for the Rapid Liquid Chromatographic Separation of Substance Mixtures and for the Identification of Substances
Applicant(s) for DO/EO/US:

Applicant hereby claims Small Entity Status pursuant to 37 F.R. §1.27

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items under 35 U.S.C. 371:

- ☒ This express request to immediately begin national examination procedures (35 U.S.C. 371(f)).
- ☒ The U.S. National Fee (35 U.S.C. 371(c)(1) and other fees as follows:

TOTAL CLAIMS 7	CLAIMS OVER 20 0	RATE X \$18 =	TOTAL FEES FOR CLAIMS OVER 20 0
NUMBER OF INDEPENDENT CLAIMS 2	CLAIMS OVER 3 3	RATE X \$80 =	TOTAL FEES FOR INDEPENDENT CLAIMS OVER 3 0
MULTIPLE DEPENDENT CLAIM(S) PRESENT No	RATE \$270 per APPLN.		FEE MULTIPLE DEPENDENT CLAIM(S) 270
BASIC NATIONAL FEE (37CFR 1.492(a)(1)-(4)): — International preliminary Examination fee paid to USPTO (37 CFR 1.482) = \$690 — No International preliminary examination fee paid to USPTO (37 CFR 1.482) but — international search fee paid to USPTO (37 CFR 1.445(a)(2)) = \$710 — Neither international preliminary examination fee (37 CFR 1.482) nor International Search fee (37 CFR 1.445(a)(2)) paid to USPTO = \$1,000 — International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)(2) to (4) = \$96.00 x Filing with an EPO or JPO search report = \$860			860
Surcharge of \$130 for furnishing the national fee or oath or declaration 20 mos. from the earliest claimed priority date (37 CFR 1.482(c)).			
TOTAL OF ABOVE CALCULATIONS			1,130
Reduction by 1/2 for filing by small entity			
SUBTOTAL			565
Process fee of \$130 for furnishing the English translation later than 20 mos. from the earliest claimed priority date (37 CFR 1.482(f)).			
TOTAL NATIONAL FEE			565
Fee for recording the enclosed assignment			
TOTAL FEES ENCLOSED			

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- a. ☒ A check in the amount of \$565 to cover the above fees is enclosed.
- b. ☐ Please charge my Deposit Account No. 50-1179 in the amount of \$ to cover the above fees. A duplicate copy of this sheet is enclosed.
- c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 50-1179. A duplicate copy of this sheet is enclosed.
3. A copy of the published International Application as filed (35 U.S.C. 371(c)(2))
- a. ☒ is transmitted herewith (required only if not transmitted by the International Bureau).
- b. ☐ is not required, as the application was filed in the United States Receiving Office.
- c. ☐ has been transmitted by the International Bureau.
4. ☒ A translation of the International Application into English.
5. Amendments to the claims of the International Application under PCT Article 19
- a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
- b. ☐ have been transmitted by the International Bureau.
6. ☐ A translation of the amendments to the claims under PCT Article 19
7. ☒ An oath or declaration of the inventor [35 U.S.C. 371(c)(4)]
8. ☐ translation of the Annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).
- Other document(s) or information included:
9. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
10. ☐ An assignment document for recording. Please mail the recorded assignment document to the undersigned.
11. ☒ The above checked items are being transmitted
- a. ☐ before the 18th month publication.
- b. ☐ after publication and the Article 20 communication but before 20 months from the priority date.
- c. ☐ after 20 months (surcharge and/or processing fee included).
- Note:** Petition to revive (37 CFR 1.137(a) or (b)) is necessary if 35 U.S.C. 371 requirements submitted after 20 months and no proper demand for International Preliminary Examination was made by 19 months from the earliest claimed priority date.
- e. ☒ by 30 months and a proper demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
- f. ☐ after 30 months (surcharge and/or processing fee included).
- Note:** Petition to revive (37 CFR 1.137(a) or (b)) is necessary if 35 U.S.C. 371 requirements submitted after 32 months and a proper demand for International Preliminary Examination was made by 19 months from the earliest claimed priority date.

12. At the time of transmittal, the time limit for amending claims under Article 19
- a. ☒ has expired and no amendments were made.
- b. ☐ has not yet expired.
13. ☐ Certain requirements under 35 U.S.C. 371 were previously submitted by the applicant on _____, namely:
14. ☒ Return postcard.

Please direct all communications in connection with this application to the undersigned at

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09/807137

7/PRTS

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**Method and Device for the Rapid Liquid Chromatographic Separation of
Substance Mixtures and for the Identification of Substances**

Specification

The invention relates to a process and a device for the rapid liquid-chromatographic separation of mixtures of substances and identification of substances according to the preambles of claims 1 and 5.

For example, the pharmaceutical research frequently encounters the problem of isolating pharmaceutically active substances from mixtures of substances. Thus, extracts of natural products or mixtures of substances produced by combinatorial chemistry are being tested for potential activity. Using mixtures of substances found to have activity, attempts are then made to isolate the active substances by means of complex separation procedures. Thereafter, the individual substances of the mixture isolated in this way are subjected to an activity test once more. The active individual substances being found are investigated for their structure so as to exclude active substances which might be already well-known. One drawback in this process is that when testing mixtures of substances, the activity of individual substances may be suppressed by superposition effects, leaving these substances undetected. Another drawback is possible simulation of activity by superposition effects, followed by a cost-intensive and useless search for these supposed active substances in the mixture of substances. Ultimately, it is disadvantageous that substances already well-known are excluded only after performing at

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least two tests for biological activity and after complex isolation procedures, which is very costly. As a rule, large quantities of material are required to perform these tests, i.e., separations have to be carried out on a preparative scale. With respect to capital investment, however, preparative units are more expensive compared to analytical units. Also, preparative units use considerably higher amounts of solvents and buffer substances during separation, rendering their operation costly and, in addition, giving rise to major problems with disposal and to environmental pollution.

The invention is based on the object of providing a device and a process for the liquid-chromatographic separation, isolation and identification of substances in an analytical and semi-preparative range, by means of which testing for activity of mixtures of substances is rendered unnecessary, and which enable separating mixtures of substances and isolating and identifying the individual substances more rapidly than is possible up to now.

Said object is accomplished through the characterizing parts of claims 1 and 5.

Advantageous developments are specified in the subclaims.

The invention has various advantages. Double testing of substances, namely, beforehand in the mixture of substances and after isolation, is no longer necessary. According to the invention, the complex and costly, and in part faulty first activity test of the mixtures of substances can be omitted. Instead, following combined isolation and identification, only potentially new active substances are subjected to further tests. One can do without costly treatment of substances already well-known, as is common practice up to now. The input of time and cost for detecting a

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new active substance can be reduced substantially. In addition, this procedure is more reliable because the test results on unknown individual substances are unequivocal and also, all of the active substances present in the mixture will be detected.

The mixtures of substances to be investigated are processed in a two-stage separation wherein, because the separating columns and solid-phase extraction columns (collecting columns) are connected with the pump unit in a fashion according to the invention, parallel separation of multiple fractions from the first separation step is possible in the second chromatographic separation stage. Consequently, this apparatus works substantially more rapid and thus, more economical as compared to well-known two-stage apparatus.

The individual substances are identified using *per se* known direct computer-controlled comparison of chromatograms and spectra obtained from detectors, and of the retention range from the first separation step and the retention time from the second separation step with information on well-known substances in a data base. Ultraviolet absorption, mass spectrometry, light scattering, fluorescence, infrared spectroscopy, and nuclear magnetic resonance spectroscopy are possible as principles of detection and identification. It is also possible to use additional identification parameters such as source and origin of the sample. Because a smaller number of tests are required to identify the substances in the mixture and exclude substances already well-known, the plant can be dimensioned for analytical and semi-preparative scales. Analytical and semi-preparative units are much more economical with respect to initial cost and operation compared to preparative units commonly used so far. Owing to the reduced consumption of solvent and buffer substances, the process and device according to the

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invention are environmentally beneficial as a result of reduced amounts of waste.

The invention will be illustrated in more detail with reference to the drawings and embodiments:

- Fig. 1 is a schematic representation of the operating sequence of equilibrating in the first separation step and washing of the feed column battery;
- Fig. 2 is a schematic representation of the separation of a mixture of substances in the first separation step and the adsorption of fractions on the first collecting column battery;
- Fig. 3 is a schematic representation of the separation of a mixture of substances in the first separation step and the adsorption of fractions on the second collecting column battery;
- Fig. 4 is a schematic representation of the separation of a mixture of substances in the first separation step and the adsorption of fractions on the third collecting column battery;
- Fig. 5 is a schematic representation of the equilibration of the separation column batteries of the second separation step;
- Fig. 6 is a schematic representation of a parallel separation of adsorbed fractions in the second separation step; and
- Fig. 7 is a schematic representation of the equilibration of a collecting column battery.

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Fig. 1 through Fig. 7 representatively illustrate the configuration and flow scheme of a device according to the invention, including a separation column and three downstream separation lines.

A pump unit 2 comprised of three pumps 2.1 through 2.3 is connected via 6-way 2-position valves 3.1 and 3.3 and 3-way 2-position valve 5.7 to a feed column battery 6, a separation column 10 for the first separation stage, and a second separation stage comprised of three separation lines operable in parallel, each of which having an upstream 6-way 2-position valve 3.5, 3.6 and 3.7, respectively. In this way, it is possible to convey the mobile phase in any desired composition to any region of the device in a consecutive as well as a parallel fashion.

Each separation line has a collecting column battery 7, 8 and 9 and a separation column battery 11, 12 and 13. Representatively, the collecting column battery 7 includes the collecting columns 7.1 through 7.6, and the separation column battery 11 includes the separation columns 11.1 and 11.2. The other two illustrated separation lines are identical in configuration. Other variants including more feed columns 6.1 through 6.6 in the feed column battery 6, multiple separation columns 10, more than three collecting column batteries 7, 8 and 9, each having more than six collecting columns, and more than three separation column batteries 11, 12 and 13 having more than six separation columns per battery are also possible.

The operating sequence of the process according to the invention will be described in an exemplary fashion below. Samples of mixtures of substances are dissolved in a solvent and added with an adsorbent each time. Subsequently, the solvent is removed using a rotary evaporator so that the adsorbent covered with sample material achieves flow-

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ability properties. The adsorbents covered with the mixture of substances are filled into the feed columns 6.1 through 6.6 of feed column battery 6 and secured in feed column battery 6. The subsequent program sequence steps are controlled by a software.

The separation column 10 is equilibrated according to Fig. 1. In a parallel operation, the air is removed from the feed column battery 6. Via pump 2.3, 3-way 2-position valve 5.7 and 6-way 2-position valves 3.1 and 3.3, the air is removed with water from one of the dry-filled feed columns 6.1 through 6.6 to be injected next. At the same time, the separation column 10 is equilibrated with a suitable mobile solvent via pump 2.1 and 6-way 2-position valves 3.1 and 3.3.

Fig. 2 illustrates the separation of the mixture of substances in the first separation stage on separation column 10 and the subsequent adsorption of the fractions in a separation line including the collecting columns 7.1 through 7.6 of collecting column battery 7.

Once the air has been removed from one of the feed columns 6.1 through 6.6, the separation program is started. Initially, the 6-way 2-position valves 3.3 and 3.5 are switched in position. Via a low-pressure valve unit 1 including the low-pressure valves 1.1 through 1.3, the components of the mobile phase can be fed into the system by means of pump unit 2. The mobile phase is conveyed via the low-pressure valve 1.1 of pump 2.1 and via pump 2.1, and this system can be run both in an isocratic fashion and with a gradient. Via 6-way 2-position valve 3.3 and 7-way 6-position valves 4.1/4.2, the mobile phase is conveyed by pump 2.1 to that particular feed column 6.1 through 6.6 from which sample material is to be processed. The sample to be separated is transferred from one of the feed columns

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6.1 through 6.6 to the separation column 10. Via a 6-way 2-position valve 3.4 and detector 14.1, the separated sample components discharging from separation column 10 arrive at T-piece 17, where water is mixed into the mobile phase via pump 2.2 and 6-way 2-position valve 3.1. The amount of admixed water depends on the polarity of the substances to be separated. Now, as the polarity of the mobile is increased by the water, adsorption on collecting columns 7.1 through 7.6 of collecting column battery 7 is possible. Initially, adsorption is effected on the collecting column battery 7 via 6-way 2-position valve 3.5, the collecting columns 7.1 through 7.6 being loaded with fractions one by one.

Fig. 3 illustrates the adsorption of additional fractions on the collecting columns 8.1 through 8.6 of collecting column battery 8. Once all of the collecting columns of collecting column battery 7 are loaded with fractions, the 6-way 2-position valves 3.5 and 3.6 switch the collecting column battery 8 into the eluent stream. Now, the collecting columns 8.1 through 8.6 are loaded with fractions one by one.

Fig. 4 illustrates the adsorption of fractions on the collecting columns 9.1 through 9.6 of collecting column battery 9. Once all of the collecting columns 8.1 through 8.6 of collecting column battery 8 are loaded with fractions, the 6-way 2-position valves 3.6 and 3.7 switch the collecting column battery 9 into the eluent stream. Now, the collecting columns 9.1 through 9.6 are loaded with fractions one by one. In the next operating sequence step, the fractions adsorbed on the three collecting column batteries 7, 8 and 9 are eluted in parallel and separated further on the appropriately assigned separation column batteries 11, 12 and 13.

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The separation column batteries 11, 12 and 13 are equilibrated prior to each separation. Fig. 5 illustrates the equilibration of separation column batteries 11, 12 and 13. For equilibration, the mobile phase is conveyed to separation columns 11.1 and 11.2 of separation column battery 11 via pump 2.1 and 6-way 2-position valves 3.1 and 3.5, respectively. From there, the mobile phase is conveyed to the waste via 6-way 2-position valve 3.4, detector 14.1, and a fraction collector 15.1. In a parallel operation, the separation columns 12.1 and 12.2 of the separation column battery are equilibrated via pump 2.2, the 6-way 2-position valves 3.1 and 3.6, a detector 14.2, and fraction collector 15.2. Likewise, the separation columns 13.1 and 13.2 are equilibrated via pump 2.3, the 6-way 2-position valve 3.7 and 3-way 2-position valve 5.7, a detector 14.3, and a fraction collector 15.3 in a parallel operation.

Fig. 6 illustrates the parallel separation of fractions adsorbed on collecting column batteries 7, 8 and 9, using the separation column batteries 11, 12 and 13. To initiate the separation step, the mobile phase is conveyed to the collecting column battery 7 via pump 2.1 of pump unit 2 and 6-way 2-position valves 3.1 and 3.5. The first eluted fraction from collecting column battery 7 (e.g. from collecting column 7.1) is passed to separation column battery 11 via 6-way 2-position valve 3.5. There, one of the separation columns 11.1 or 11.2 can be switched in, optionally in a software-controlled fashion. Subsequently, the separated components are passed to the detector 14.1 via 6-way 2-position valves 3.5 and 3.4. The software in the electronic control unit assesses the signals by peak detection, directing the separated components into the appropriate vials of fraction collector 15.1. Simultaneously, time control of the fraction collector 15.1 is also possible. Such time control can be activated automatically in case no peak passes the detector.

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In a parallel operation, the mobile phase is conveyed to the collecting column battery 8 via pump 2.2 and 6-way 2-position valves 3.1 and 3.6. The first eluted fraction from collecting column battery 8 (e.g. from collecting column 8.1) is passed to separation column battery 12 via 6-way 2-position valve 3.6. There, one of the separation columns 12.1 or 12.2 can be switched in, optionally in a software-controlled fashion. The separated components are passed to the detector 14.2. In this case as well, the software assesses the signals by peak detection, subsequently directing the separated components into the appropriate vials of fraction collector 15.2. Similarly, the fraction collector 15.2 can be operated in a time-controlled fashion. Such time control can be activated automatically in case no peak passes the detector.

In parallel to the operations in two separation lines, the third separation line is activated with respect to initiating the separation step. To this end, the mobile phase is passed to collecting column battery 9 via pump 2.3 and 3-way 2-position valve 5.7 and 6-way 2-position valve 3.7. The first eluted fraction from collecting column battery 9 (e.g. from collecting column 9.1) is passed to separation column battery 13 via valve 3.7. There, one of the separation columns 13.1 or 13.2 can be switched in, optionally in a software-controlled fashion. The separated components are passed to the detector 14.3. The downstream fraction collector 15.3 is controlled as described above. After each one of the first fractions has been processed in parallel, the separation column batteries 11, 12 and 13 are re-equilibrated (cf., Fig. 5) for preparation and in order to separate the next fractions. Subsequently, the 7-way 6-position valves 4.3/4.4, 4.5/4.6 and 4.7/4.8 on the collecting column batteries 7, 8 and 9 are switched over, so as to enable processing of the second fractions as illustrated in

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Fig. 6. These operations are continued until all of the fractions have been processed.

Fig. 7 illustrates the equilibration of the collecting columns 7.1 through 7.6 of collecting column battery 7. In this program sequence step, the collecting columns 7.1 through 7.6 are washed with water, thereby being prepared for the next cycle. This is done in a sequential fashion via pump 2.2, 6-way 2-position valves 3.1, 3.5, 3.6, 3.7, and 7-way 6-position valves 4.3/4.4 of collecting column battery 7. Equilibration of the collecting column batteries 8 and 9 is performed in an analogous manner. The 6-way 2-position valves 3.5 and 3.6 are switched in position, and the collecting columns 8.1 through 8.6 are equilibrated via pump 2.2, 6-way 2-position valves 3.1, 3.5, 3.6, 3.7, and 7-way 6-position valves 4.5/4.6 of collecting column battery 8. Subsequently, the 6-way 2-position valves 3.6 and 3.7 are switched in position, and the collecting columns 9.1 through 9.6 are equilibrated via pump 2.2, 6-way 2-position valves 3.1, 3.5, 3.6, 3.7, and 7-way 6-position valves 4.7/4.8 of collecting column battery 9. Following this program sequence, the 7-way 6-position valves 4.1/4.2 of feed battery 6 are switched over to the next feed columns (e.g. 6.2), and the entire program cycle is restarted (Sequence step 1: Equilibration of separation column 10 and venting of feed column 6.2, illustrated in Fig. 1, etc.).

After processing the second sample, the next feed column 6.3 can be switched in the eluent stream. Because sample feed columns having undergone processing can be replaced by new ones anytime, continuous operation with an unlimited number of samples is possible.

During the first and second separation steps, chromatograms, retention data and spectra are collected via detectors 14.1, 14.2 and 14.3, processed directly in a com-

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puter, and compared with data of known substances. Thus, known substances can be identified and sorted out already in on-line mode. In doubtful cases, additional data obtained off-line following separation and isolation can be used for identification.

Reference list

- 1 Low-pressure valve unit
- 1.1 Low-pressure valve
- 1.2 Low-pressure valve
- 1.3 Low-pressure valve
- 2 Pump unit
- 2.1 Pump
- 2.2 Pump
- 2.3 Pump
- 3 6-way 2-position valve
- 3.1 6-way 2-position valve
- 3.3 6-way 2-position valve
- 3.4 6-way 2-position valve
- 3.5 6-way 2-position valve
- 3.6 6-way 2-position valve
- 3.7 6-way 2-position valve
- 4 7-way 6-position valve
- 4.1 7-way 6-position valve
- 4.2 7-way 6-position valve
- 4.3 7-way 6-position valve
- 4.4 7-way 6-position valve
- 4.5 7-way 6-position valve
- 4.6 7-way 6-position valve
- 4.7 7-way 6-position valve
- 4.8 7-way 6-position valve
- 5 3-way 2-position valve
- 5.1 3-way 2-position valve
- 5.2 3-way 2-position valve
- 5.3 3-way 2-position valve
- 5.4 3-way 2-position valve
- 5.5 3-way 2-position valve
- 5.6 3-way 2-position valve
- 5.7 3-way 2-position valve

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6 Feed column battery
5.1 Feed column
5.2 Feed column
5.3 Feed column
5.4 Feed column
5.5 Feed column
5.6 Feed column
7 Collecting column battery
7.1 Collecting column
7.2 Collecting column
7.3 Collecting column
7.4 Collecting column
7.5 Collecting column
7.6 Collecting column
8 Collecting column battery
8.1 Collecting column
8.2 Collecting column
8.3 Collecting column
8.4 Collecting column
8.5 Collecting column
8.6 Collecting column
9 Collecting column battery
9.1 Collecting column
9.1 Collecting column
9.2 Collecting column
9.3 Collecting column
9.4 Collecting column
9.5 Collecting column
9.6 Collecting column
10 Separation column
11 Separation column battery
11.1 Separation column
11.2 Separation column
12 Separation column battery
12.1 Separation column
12.2 Separation column

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- 13 Separation column battery
- 13.1 Separation column
- 13.2 Separation column
- 14 Detectors
- 14.1 Detector
- 14.2 Detector
- 14.3 Detector
- 15 Fraction collector
- 15.1 Fraction collector
- 15.2 Fraction collector
- 15.3 Fraction collector
- 16 Waste
- 16.1 Waste
- 16.2 Waste
- 17 T-piece

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Claims:

1. A process for the rapid liquid-chromatographic separation and identification of substances, characterized in that mixtures of substances are pre-separated in the first stage of a software-controlled rapid liquid-chromatographic two-stage separation, the pre-separated fractions deposited in collecting columns are subjected to a parallel fine separation in at least two separation lines in the second stage, and the finely separated fractions are identified and isolated in parallel operations each time.
2. The process according to claim 1, characterized in that the pre-separation of mixtures of substances is performed consecutively in the first separation stage, and fine separation in the second stage is performed consecutively and/or in parallel.
3. The process according to claim 1 or 2, characterized in that at least one detector (14.1) is used both after the first separation stage and after the second separation stage.
4. The process according to any of claims 1 to 3, characterized in that the substances separated and isolated in the separation lines are subjected to an additional purification procedure, particularly an adsorptive purification.
5. A device for the rapid liquid-chromatographic separation and identification of substances, said device consisting of a plurality of separation columns and col-

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lecting columns, as well as feed systems, detectors, and fraction collectors, the interaction of which can be controlled through a central control unit, characterized in that a plurality of parallel liquid-chromatographic separation lines, each one consisting of a combination of separation column batteries (11, 12, 13) and collecting column batteries (7, 8, 9), detector units (14), and fraction collector units (15), are arranged downstream of at least one separation column (10), that a pump unit (2) consisting of three pumps (2.1, 2.2, 2.3) to convey the mobile phase is functionally connected both with the separation column (10) and the separation lines, and that software-switchable multi-way valves are arranged between the individual functional units.

6. The device according to claim 5, characterized in that each separation line has a multi-way valve (3.5, 3.6, 3.7) arranged upstream thereof.
7. The device according to claim 5 or 6, characterized in that additional collecting columns are arranged downstream of the separation lines.

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Abstract:

The invention relates to a process and a device for the rapid liquid-chromatographic separation of mixtures of substances and identification of substances.

The invention is based on the object of providing a device and a process for the liquid-chromatographic separation, isolation and identification of substances in an analytical and semi-preparative range, by means of which testing for activity of mixtures of substances is rendered unnecessary, and which enable separating mixtures of substances and isolating and identifying the individual substances more rapidly than is possible up to now.

Said object is accomplished by means of a process and device, wherein mixtures of substances are pre-separated in the first stage of a software-controlled rapid liquid-chromatographic two-stage separation, the pre-separated fractions deposited in collecting columns are subjected to a parallel fine separation in at least two separation lines in the second stage, and the finely separated fractions are identified and isolated in parallel operations each time (Fig. 1).

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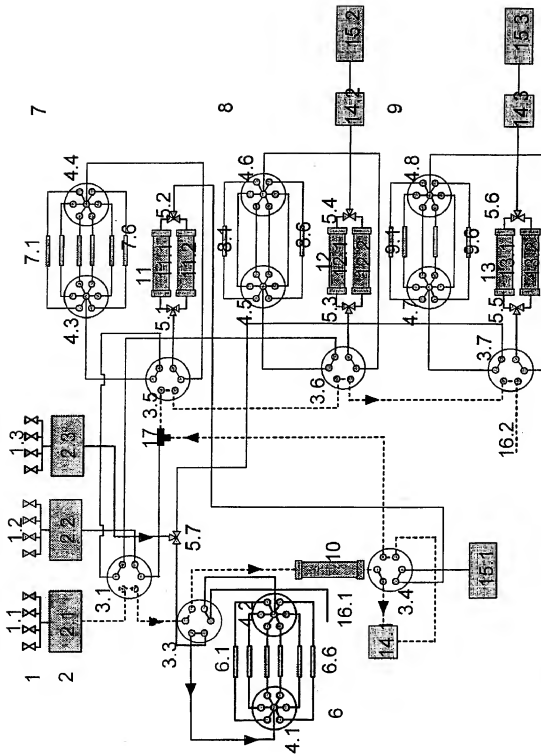


FIG. 1

ERSATZBLATT (REGEL 26)

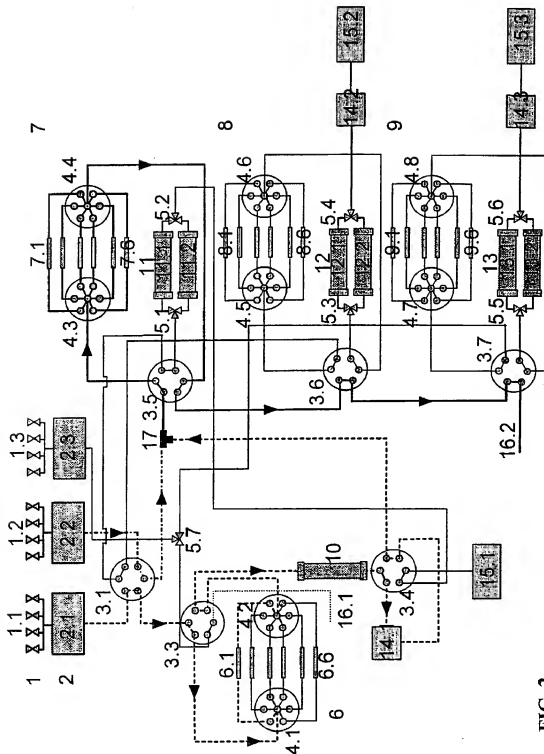


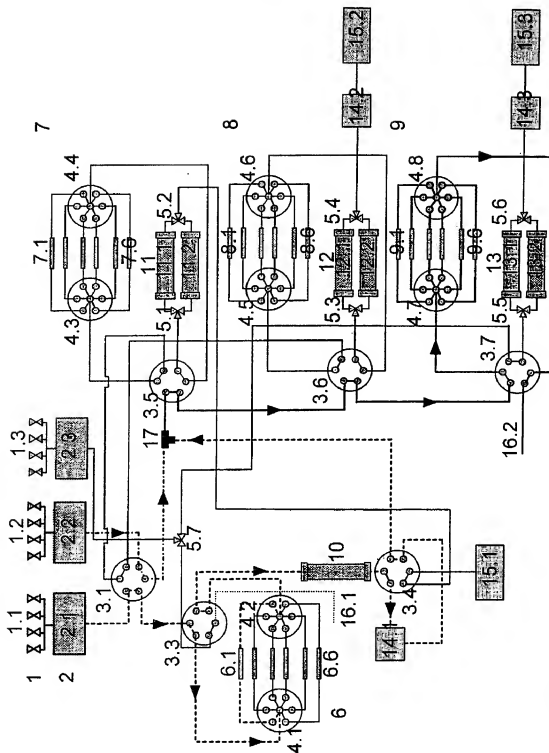
FIG. 2

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ERSATZBLATT (REGEL 26)

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**ERSATZBLATT (REGEL 26)**

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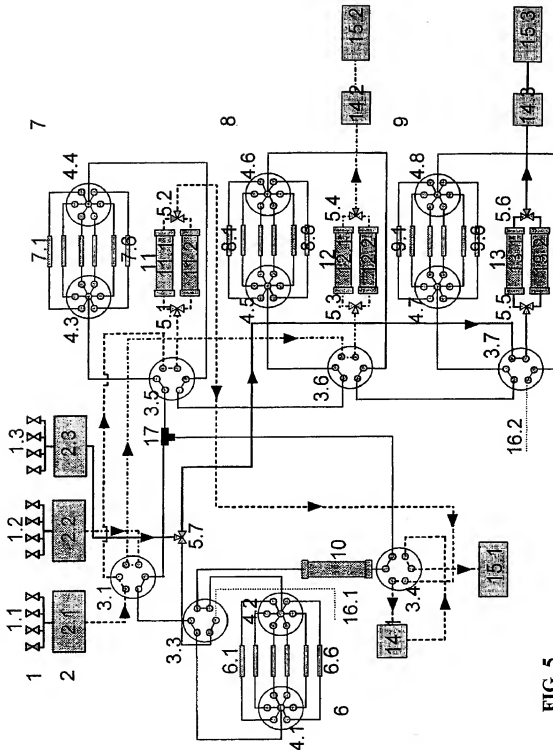


FIG. 5

ERSATZBLATT (REGEL 26)

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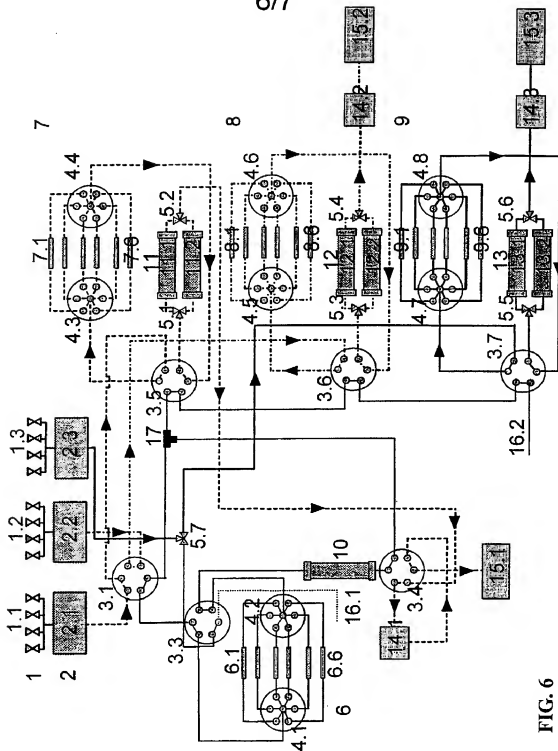


FIG. 6

ERSATZBLATT (REGEL 26)

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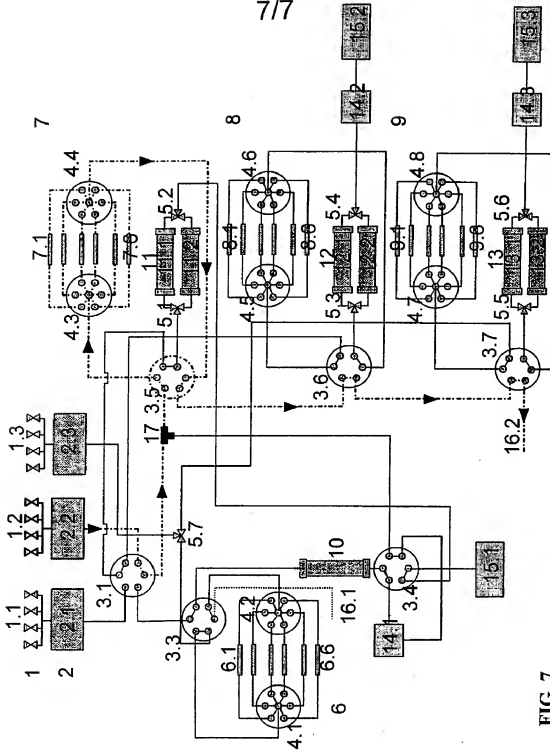


FIG. 7



09807137.120902 #6

BEROLD AND HAINES, P.A.
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United States of America

If each inventor understands English, the Declaration and Power of Attorney below is suitable for use when filing a regular patent application and also when entering the national stage, in the case of an International application designating the USA under the PCT.

COMBINED DECLARATION AND POWER OF ATTORNEY FOR PATENT APPLICATION			Attorney Docket no 33148.004.																						
<p>As a below named inventor, I hereby declare that: My residence, post office address and citizenship are as stated below next to my name, I believe I am the original, first and sole inventor (if only one name is listed below at 201) or an original, first and joint inventor (if plural names are listed below at 201-205) of the subject matter which is claimed and for which a patent is sought on the invention entitled</p> <p>Method and Device for the Rapid Liquid Chromatographic Separation of Substance Mixtures and for the Identification of Substances</p> <p>the specification of which (check one)</p> <p>_____ is attached hereto</p> <p><input checked="" type="checkbox"/> was filed on <u>8 October 1999</u></p> <p>under Serial Number <u>PCT/EP99/07542</u> and was amended on _____ (if applicable).</p> <p>I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.</p> <p>I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, Section 1.56.</p> <p>I list below any prior foreign application(s) for patent or inventor's certificate in respect of which foreign priority benefits are claimed under 35 USC 119; and any prior foreign application(s) for patent or inventor's certificate in respect of which such foreign priority rights are not claimed and which has a filing date before that of any application in respect of which such foreign priority benefits are claimed:</p> <table border="1"><thead><tr><th>Application Number</th><th>Country</th><th>Filing Date (day, month, year)</th><th>Priority Claimed under 35 USC 119</th></tr></thead><tbody><tr><td>198 47 439.3</td><td>GERMANY (DE)</td><td>08.10.99</td><td>YES: <input checked="" type="checkbox"/> NO: _____</td></tr><tr><td></td><td></td><td></td><td>YES: _____ NO: _____</td></tr><tr><td></td><td></td><td></td><td>YES: _____ NO: _____</td></tr></tbody></table> <p>I hereby claim the benefit under Title 35, United States Code, §119(e) of any United States provisional application(s) listed below.</p> <table border="1"><thead><tr><th>Application No.</th><th>Filing Date</th></tr></thead><tbody><tr><td></td><td></td></tr><tr><td></td><td></td></tr></tbody></table>				Application Number	Country	Filing Date (day, month, year)	Priority Claimed under 35 USC 119	198 47 439.3	GERMANY (DE)	08.10.99	YES: <input checked="" type="checkbox"/> NO: _____				YES: _____ NO: _____				YES: _____ NO: _____	Application No.	Filing Date				
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I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

Brian L. Wamsley (33,045)

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signature of Inventor 201	<i>[Signature]</i>	Date	<i>April 10, 2001</i>
Signature of Inventor 202	<i>[Signature]</i>	Date	<i>June 10, 2001</i>
Signature of Inventor 203	<i>[Signature]</i>	Date	<i>June 12, 2001</i>
Signature of Inventor 204	<i>[Signature]</i>	Date	<i>April 13, 2001</i>
Signature of Inventor 205		Date	